



Recurrent Mutation and Genome Evolution: Example of *Sugary 1* and the Origin of Sweet Maize

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Abstract

In maize (*Zea mays* L.), mutations at the *Sugary 1* locus are the genetic bases for maize with specialty uses cultivated throughout the western hemisphere precontact (pre-Columbian). The traditional North American sweet maize is homozygous for a recessive *sugary1* (*su1*) allele. Determining the number of unique alleles among *su1* maize races is relevant to the debate in archeology and evolutionary biology over whether independent mutation or migration plays a dominant role in the spread of novel phenotypes. We sequenced *su1* from 57 cultivars of *su1* maize and determined that five independent mutations have been selected. Three of these five alleles were single base pair changes at highly conserved sites and a fourth was a 1.3-kbp transposon. It will be interesting to note if future study in a variety of disciplines will lead to consensus on the significant role of recurrent mutation in evolution.

“SEX IS GOOD, but not as good as fresh sweet corn.”

When American humorist Garrison Keillor made this now famous remark he probably was not aware that his indebtedness is owed to a recessive allele at *Sugary 1* (*su1*). Maize homozygous for recessive *su1* has been cultivated in much of the maize growing region of the western hemisphere since precontact times (Wellhausen et al., 1952; Grobman et al., 1961). The gene is now known to code for an isoamylase functioning in starch synthesis in maize endosperm (Rahman et al., 1998; Dinges et al., 2001). Recessive *su1* results in the accumulation of phytyglycogen rather than starch (Marshall and Tracy, 2003). Phytyglycogen, a highly branched water soluble polysaccharide, gives sweet maize its creamy texture (Marshall and Tracy, 2003).

In 1890 Willet M. Hays (1890, p. 89–90) anticipated Mendel's laws by reporting the phenomena of dominance, recessiveness, segregation, and independent assortment by clearly describing the 3:1 segregation ratio in controlled crosses of flint maize and *su1* maize. Alleles at *Su1* were among the first to be genetically characterized by Correns (1901) following the rediscovery of Mendel. While *su1* was one of the earliest genes genetically characterized in maize, maize geneticists have long debated its origins. The controversy has centered on whether one or two independent mutations at *Su1* have been selected and fixed. Galinat (1971) and Mangelsdorf (1974) used morphological evidence

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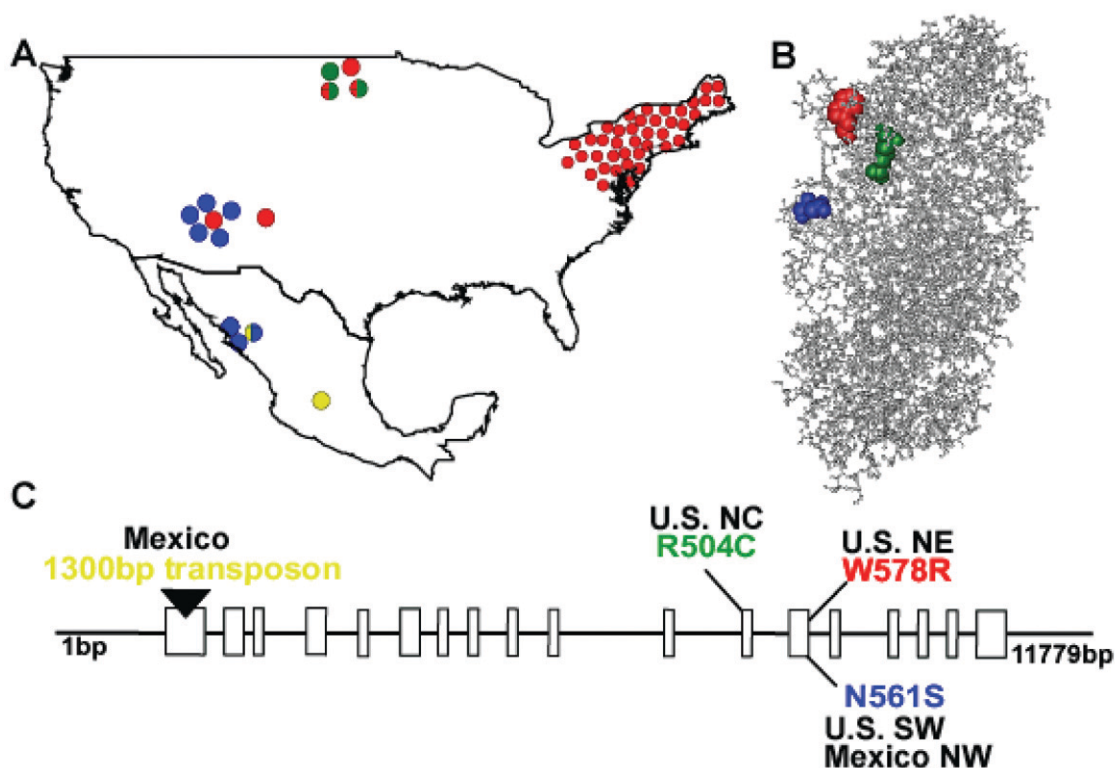


Fig. 1. Distribution of *su1* alleles. Alleles are color coded based on (C). (A) The geographic distribution of *su1* mutant alleles. Mixed accessions are indicated by split circles. Exact geographic locations for the majority of the northeastern U.S. accessions are unknown. (B) The three *su1* amino acid changes projected on *Pseudomonas* isoamylase crystal structure [Katsuya et al., 1998]. (C) Structure of the *Su1* gene and position of mutations. A fifth allele, from the Peruvian accessions, is not depicted in this figure.

to argue that the *su1* allele was selected once in the Peruvian Andes and then introgressed into local maize throughout the hemisphere. In this hypothesis (Mangelsdorf, 1974), the Peruvian race Chullpi was the original *su1* source and the progenitor of the race Maiz Dulce from Jalisco, Mexico. Maiz Dulce, in turn, was crossed to popcorn Reventador to produce Ducillo del Noroeste in northwest Mexico (Wellhausen et al., 1952). From Ducillo del Noroeste, *su1* was introgressed into northern races including Northern Flint, the progenitor of modern commercial sweet corn (Revilla and Tracy, 1995).

Others counter that independent mutations at *su1* were fixed twice—first, thousands of years ago in the Andes, and again more recently in what is now the northeastern USA (Erwin, 1934, 1947, 1951). This theory is based on three main arguments: (i) lack of sweet maize in archeological collections from eastern North America, (ii) observations of spontaneous mutations to the *su1* allele in field maize, and (iii) the fact that no written record of sweet maize exists in the USA until the nineteenth century. The earliest written English references to what is indisputably *su1* corn, based on its distinctive wrinkled seed, appeared in 1801 in a book called Bordley's Husbandry (Stur-

tevant, 1972) and 1810 in Thomas Jefferson's Garden Book (Huelson, 1954). Thus, the dispute over the origins of *su1* maize fuels the larger debate in archeology and evolutionary biology over whether independent mutation or migration plays a more dominant role in the spread of novel phenotypes.

To help resolve this controversy, we sequenced the *Su1* gene from 57 accessions of *su1* maize and compared the results with normal *Su1* alleles identified in a previous study (Whitt et al., 2002) (Fig. 1A and Table 1).

Materials and Methods

Seed from 57 *sugary1* accessions encompassing six geographic areas in the USA, Mexico, and Peru were obtained from the North Central Plant Introduction Center in Ames, IA, and the University of Wisconsin sweet corn breeding program (Table 1). Seed were germinated and leaf tissue harvested from two plants per accession at 14 d. DNA was extracted in a 96-well format from frozen tissue. Samples were PCR amplified and sequenced at the *SU1* locus. Two 1000-bp regions of the gene with previously identified sweet mutations, an exon 1 transposable element found in a sample from Jalisco, Mexico (Whitt et al.,

Table 1. Fifty seven *sugary1* maize accessions, their immediate source, and the putative race and area of origin.

Accessions	Source [†]	Putative race or group	Putative area of origin	Mutation
Apurimac 37	PI 571870	Chulpi	Peru	unknown
Apurimac 95	PI 571586	Chulpi	Peru	unknown
Guanajuato 181	PI 628428	Maiz Dulce	Guanajuato, Mexico	1.3-kbp transposable element in exon 1
Z07-011	PI 503576	Maiz Dulce	Sinaloa, Mexico	1.3-kbp transposable element in exon 1/N561S
Ducillo de Noroeste	PI 474214	Ducillo de Noroeste	Sonora, Mexico	N561S
Ducillo de Noroeste	PI 474215	Ducillo de Noroeste	Sonora, Mexico	N561S
Ducillo de Noroeste	PI 474213	Ducillo de Noroeste	Sonora, Mexico	N561S
Hopi 15 Lt. Red Striped	NSL 67056	Southwestern 12 Row	southwestern USA	W578R
Hopi White	WI:94:4430	Southwestern 12 Row	southwestern USA	N561S
Hotevilla	WI:99:5732	Southwestern 12 Row	southwestern USA	N561S
Monacopi Pueblo	PI 218174	Southwestern 12 Row	southwestern USA	N561S
Tawa'ktci	PI 420247	Southwestern 12 Row	southwestern USA	N561S
Tawaktei	PI 476873	Southwestern 12 Row	southwestern USA	N561S
Tesuque Pueblo	PI 218134	Southwestern 12 Row	southwestern USA	W578R
Hidatsa	WI:93:9717	Great Plains Flints and Flours	northcentral USA	W578R
Mandan Red	WI:93:943	Great Plains Flints and Flours	northcentral USA	R504C/W578R
Nueta	PI 219886	Great Plains Flints and Flours	northcentral USA	R504C
Nueta Sweet Corn	PI 213796	Great Plains Flints and Flours	northcentral USA	R504C/W578R
Alta Gold	PI 219870	Northern Flint	northeastern USA	W578R
Baby Orchard	PI 219872	Northern Flint	northeastern USA	W578R
Bantam Evergreen	WI:93:8925	Northern Flint	northeastern USA	W578R
Baxter's Golden Bantam	PI 255982	Northern Flint	northeastern USA	W578R
Black Aztec	WI:93:8929	Northern Flint	northeastern USA	W578R
Black Mexican	PI 162573	Northern Flint	northeastern USA	W578R
Buhl	WI:94:4420	Northern Flint	northeastern USA	W578R
Campbell	Ames 22638	Northern Flint	northeastern USA	W578R
Dorinny	WI:96:6811	Northern Flint	northeastern USA	W578R
Early Pearl	PI 228183	Northern Flint	northeastern USA	W578R
Golden Bantam	PI 219879	Northern Flint	northeastern USA	W578R
Golden Bantam	PI 255976	Northern Flint	northeastern USA	W578R
Golden Bantam (MA)	PI 255977	Northern Flint	northeastern USA	W578R
Golden Bantam (WI)	WI:93:8721	Northern Flint	northeastern USA	W578R
Golden Early Market	WI:93:9314	Northern Flint	northeastern USA	W578R
Golden Gem	PI 219880	Northern Flint	northeastern USA	W578R
Golden Sunshine	Ames 22641	Northern Flint	northeastern USA	W578R
Hayes White	WI:93:9618	Northern Flint	northeastern USA	W578R
Hooker's Sweet Indian	WI:93:9132	Northern Flint	northeastern USA	W578R
Kennedy's White Midget	WI:93:9518	Northern Flint	northeastern USA	W578R
Lindsey Meyer Blue	WI:00:6422	Northern Flint	northeastern USA	W578R
Luther Hill	WI:93:9015	Northern Flint	northeastern USA	W578R
Midnight Blue	WI:93:9124	Northern Flint	northeastern USA	W578R
Midnight Snack	WI:93:9021	Northern Flint	northeastern USA	W578R
No Name	WI:93:9126	Northern Flint	northeastern USA	W578R
Orchard Baby	WI:93:9231	Northern Flint	northeastern USA	W578R
Pease Crosby	PI 255983	Northern Flint	northeastern USA	W578R
Queen Anne	WI:93:9228	Northern Flint	northeastern USA	W578R
Stowell's Evergreen	WI:93:8819	Northern Flint	northeastern USA	W578R
Sunshine	PI 219894	Northern Flint	northeastern USA	W578R
Sweet Baby Blue	WI:96:6831	Northern Flint	northeastern USA	W578R
West Brookfield White	PI 255975	Northern Flint	northeastern USA	W578R
Whipple's Yellow	PI 231301	Northern Flint	northeastern USA	W578R
Whipple's Yellow	WI:93:9416	Northern Flint	northeastern USA	W578R
Yukon Chief	WI:97:10539	Northern Flint	northeastern USA	W578R
Country Gentleman	WI:01:8861	unknown	unknown	W578R
Early June	PI 219876	unknown	unknown	W578R
Malcomb's	WI:93:9017	unknown	unknown	W578R
Red	WI:94:4450	unknown	unknown	W578R

[†] Ames, NSL, and PI designations refer to stocks obtained from the North Central Regional Plant Introduction Station, Ames, IA. Accessions with a WI designation are from the collection maintained by the Department of Agronomy, University of Wisconsin, Madison.

2002), and a tryptophan to arginine substitution at residue 578 in northeastern U.S. sweet corn (Dinges et al., 2001; Whitt et al., 2002) were initially examined.

Nucleotide alignments were generated using Biolign alignment software (Thomas Hall, 2000, North Carolina State University). Twenty-nine accessions of nonsweet maize were included in the alignments to enable detection of polymorphisms unique to sweet maize. Representative accessions from each region demonstrating a novel mutation were PCR amplified and sequenced for the entire *SU1* locus. The accessions sequenced were PI 474214 (Ducillo de Noroeste, Sonora, Mexico), WI:93:943 ('Mandan Red'), and PI 213796 ('Nueta', northcentral USA), and WI:94:4430 ('Hopi White', southwestern USA). No additional polymorphisms were discovered in a nucleotide alignment of the 11 kbp *su1* gene when compared with 32 completely sequenced maize inbred lines.

Results and Discussion

Our survey revealed five independent origins in the history of sweet maize, four of which were identified to the nucleotide level (Fig. 1C, Table 1). All 35 of the northeastern USA cultivars we sequenced had a tryptophan to arginine substitution at residue 578 (W578R). These are the progenitors of modern commercial sweet maize (Revilla and Tracy, 1995; Gerdes and Tracy, 1994). The four cultivars of unknown race and geographic origin also had the W578R allele. These results are similar to those reported by Whitt et al. (2002), and this W578R allele was also one of two identified by Dinges et al. (2001).

Three Ducillo de Noroestes accessions from northwest Mexico and five of the seven accessions from southwestern USA had an asparagine 561 to serine mutation (N561S), indicating a second event. The two remaining southwestern USA accessions carried the W578R allele. This finding may represent the origin of this allele in what is now Arizona or New Mexico, or perhaps signal a more recent immigration of the allele to this area.

A third independent event was confirmed by the 1.3-kbp transposable element in exon 1 found in Maiz Dulce accession from Guanajuato, Mexico (PI 628428). This is the same transposable element previously observed in Mexican *su1* maize (Whitt et al., 2002). The other Maiz Dulce accession (PI 503576) segregated for both the transposable element and the N561S alleles. PI 503576 may be derived from a cross between a Maiz Dulce and a Ducillo de Noroeste. PI 503576 has morphological resemblance to Maiz Dulce, but was collected in a region more typical of the

adaptive zone for Ducillo de Noroeste (www.ars-grin.gov, verified 26 May 2006). PI 503576 was collected at an altitude of 190 m in northern Sinaloa (26°25' N, 108°31' W), less than 100 km from where one of the Ducillo de Noroeste accessions (PI 474214) was collected in the state of Sonora (27° N, 109° W). The relatively low altitude at which PI 503576 grows is more typical of Ducillo de Noroeste than the midaltitude adapted Maiz Dulce (1000–1500 m).

A fourth event most likely occurred in northcentral USA. Three of the four accessions believed to be from this region were characterized by an arginine to cysteine mutation at residue 504 (R504C), with the fourth (Hidatsa) possessing the W578R allele. Two of the three accessions were heterozygous for W578R and R504C.

Finally, despite sequencing 12 kb of *Su1* in two Chullpi accessions from the Andes, we were unable to identify a mutation that causes the *su1* phenotype. However, as the four alleles described above were not present, a fifth independent origin is apparent. Complementation tests demonstrate that the glassy wrinkled endosperm of Chullpi is due to an allele at *su1*.

Of the five mutation events, the three single amino acid substitutions are found in highly conserved residues. A comparison of the 25 isoenzymes among plants, archaea, and eubacteria revealed that residue 504 is conserved in 92% of the homologs, residue 561 in 100%, and residue 578 in 84% (Fig. 2). Interestingly, all three residues reside within a single cleft of the enzyme, away from the centrally embedded active site (Fig. 1B).

With at least five independent mutations selected and fixed by agriculturalists in American sugary maize, the multiple origins of *su1* provide a clear example of the importance of reinvention in the evolution of unique crops. This stands in sharp contrast to the emphasis previously placed on long range migrations to explain the presence of sweet maize across the Americas. Both historical and archeological studies support the idea that the Chullpi allele, the Maiz Dulce allele from central Mexico containing the 1.3-kbp transposon, and the N561S allele from northwestern Mexico/southwestern USA occurred precontact. Most probably, the northcentral R504C allele originated in the northern Great Plains sometime after maize was introduced into that region. While it is likely the northeastern W578R allele was selected in the northeastern USA between 200 and 300 yr ago, it could have originated in either the northcentral or southwestern regions and migrated to the northeast.

These *su1* alleles have a major phenotypic effect that is easily identifiable by humans. It will be interesting to learn if future investigation confirms the role of recurrent mutation in evolution occurs

	R504C		N561S	W578R
Maize (<i>Zea mays</i>)	VWSEWNGKYRDTVRQFIKGT---DGFAGAFAECLCGSPQLYQAGGRKPWH		SIGFVCAHDGFTLADLVITYNSKYNLSNGEDF-RDG-ENHNLSWNCGEEGE	
Rice (<i>Oryza sativa</i>)	I.....I.....G.....H.....		..N.....K...S.....N.....	
Barley (<i>Hordeum vulgare</i>)I.....G.....		..N.....HN...P.....N.....	
Arabidopsis thaliana	I.....F..V.....S.....N.....		..N.I.....N.N...A...EN.N.....Y.....D	
Arabidopsis thaliana	R.A...M...D...R...D...S.MK.S..TRVS..SD...VNQ...Y.		GVN..I.....R...S...F.H.EA...GG.N...C.D.H...F...	
Synechocystis sp.	R.A.....K...D...A.VI.EM.QR.Q...D...GA..P.ST		..N..T.....A..G.H.YA...NG.N...A.D.Y...V..P	
Synechocystis sp.	WFA...PF..D...R.V..D...N.AVP.L.SR.L...DI.YRQDIDNR		..N..TC.....V...S...E.H.EA...KN.....T.D.F...V...	
Deinococcus radiodurans	N.A....I...DM.S.W..E...G.L.SEIGYRIT..SD..EFN....YA		..N..T.....R.S...EQ.H.EA...GN.N...H...IT...V..P	
Arthrobacter sp. Q36	Q.T.....D.WR.E...PATL.E..SRIT..AD..EHS..R.VA		..N..T.....R...S...E.H.EA...N.K...S..R.....V..P	
Sulfolobus acidocaldarii	L.A.....I.R.WR.D...PVPYEEL.NR.L...D..AGSNKT.FA		..NYITS.....Q...S...Q.H.EA.KLNN.E...M.E.Y...V...	
Sulfolobus solfataricus	Q.A.....SI.R.WR.E...ALPYSEI.NR.L...DI.LGNNKT.FA		..NY.TS.....E...S...Q.H.EA..FNN.Q...M.E.Y...A..P	
Mycobacterium tuberculo	L.T.....DYWR.E...PATL.E..SR.T..SD..E.T..R.SA		..N..T.....N...S...D.H.EA...NN.....SY.R...V..P	
Streptomyces coelicolor	L.....A..D.WRAE...HSL.E..SR.T..SD...HSR.R.RA		.VN..T.....R...S...D.H.EA...N.....S..R...A..G	
Streptomyces coelicolor	L.T...DR..NA..D.WRHA...LPDVREMGYR.S..SD..AW...R.YA		.VN..T.....R...S..ER.H.EA...GN.....TDD.R...T...	
Pseudomonas aeruginosa	G.A...DRF..C..AYWR.D...MLPEL.RR.TA.GD..DQR..R.YA		.VN..T.....R.V.S.DH.H.EA...NN.A...SD.....H.C..P	
Escherichia coli	LFA...DHF..AA.R.WLHY...LPL...GRFAA.SDVFKRN..L.SA		A.NL.T.....R.C.CF.H.H.EA...EN.....T.N.Y.N.H.K..L	
Vibrio cholerae	-N.T.D.L..IT.S.WR.D...L.LKE..TR.M..RD..S.ANWPYKL		TVNYITY.....Q...S.KH.H.EA...QN.....HGD.R.D.Y.F..D	
Rhizobium tropici	PFL...DRA..DL.R.WR.-...AGI.DL.TI.A..SSIFGRD..TQTR		CVN.LA.....M...S.EN.H.EA...NN.....H.E.S...N.I..K	
Flavobacterium sp.	G.A....L...AL.KKQNKLGVEVTP.TL.TRFA..ND..GDD.....		..N..V.....N..YA..D.Q.NQPWPYGPS..G.D.....Q-----	
Myroides odoratus	G.A....A...V...AQNKLGSAITT.QM.TRFA..SD..GDD.....		.VN.IT.....K..YSC...N.NQVWPYGPS..G.DN.N..DQ-----	
Pseudomonas sp.	G.....LF..SL..AQNELGSMTIYVTQD.NDFS..SN.F.SS..S..N		..N.IDV...M..K.VYSC.GAN.SQAWPYGPS..GTST.Y..DQ.MS-A	
Pseudomonas sp.	RVVRVWSVPRQL..AQNELGSMTIYVTQD.NDFS..SN.F.SS..S..N		..N.IDV...M..K.VYSC.GAN.S-QASYGPS..GTST.Y..DQ.MS-A	
Haemophilus influenzae	YFA...DRF..DLCR.WLWK...S.EI....RFA..SD.FKKND.L.HT		TLN.IT.....K...S...Q.H.ET...EN.....R.E.Y.Y.H.V..S	
Chlamydomonas pneumonia	R.....P...N.KA.LN.D...QNLI.T..SRIS..QDI.P--HGS.TN		..NY.SC.....C.T...H.H.EA...N.....TDA.Y.Y.F.T..K	

Fig. 2. Alignment of isoamylases surrounding the three amino acid mutations found in maize *su1*.

primarily when alleles have a very large phenotypic effect, as well as whether or not the role of recurrent mutation in crop evolution and domestication is currently underestimated.

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